

## Letters to the Editor

### Complementarities and Convergence of Results in Bacteriorhodopsin Trimer Simulations<sup>‡</sup>

The results published by Kandt, Schlitter, and Gerwert in a recent issue of *Biophysical Journal* (Kandt et al., 2004) on molecular dynamics (MD) simulations of bacteriorhodopsin (bR) trimer in a water/lipid environment represent a new and valuable step in the field of bacteriorhodopsin modeling and, more generally, in the developing field of simulations of fully integrated and functional membrane biosystems. In their work, Kandt et al. report on water dynamics in and around bacteriorhodopsin trimer as a function of time and as a function of the protonation state of the retinal moiety. Their findings complement our previously published results on simulation of bR in monomers as well as of the purple membrane (PM), comprising bacteriorhodopsin trimers explicitly hydrated in their complete native functional lipid environment (Baudry et al., 2001). The two studies exhibit differences in technical and structural respects in the type of lipids (POPC bilayer in Kandt et al., native PM lipids in Baudry et al., including squalene molecules, the removal of which leads to modified photocycle kinetics), in the force field (GROMACS in Kandt et al., CHARMM in Baudry et al.), in simulation time (5 ns in Kandt et al., 1 ns in Baudry et al.), in the MD engine used (GROMACS in Kandt et al., NAMD2 for MD simulations and CHARMM for free energy calculations in Baudry et al.), as well as in the starting structures for ground-state bR. Despite these differences, we find it extremely interesting that several of the findings previously published in Baudry et al., in particular the role of Asp-96, Arg-82, and retinal isomerization on internal water movement and water exchange with the bulk, are very similar to those reported in Kandt et al. As the results of Baudry et al. were not cited nor commented on in Kandt et al., we believe it is of interest to discuss the similarities of the two articles in this letter to exemplify the convergence, reliability, and maturity of recent advances in the field of bR molecular modeling.

#### CONFORMATIONAL CHANGES OF ARG-82 AND ITS EFFECT ON WATER MOVEMENT

The hydration pattern around retinal is similar in both Kandt et al. and Baudry et al., in particular, with water molecules 401, 402, 406, and 407 present in both models in the retinal/Asp-85/Asp-212/Arg-82 region.

The results published in Baudry et al. on bR monomer simulations indicated that water rearrangement could take place when the retinal changes its isomeric state. Water rearrangement, connecting the Asp-85/Asp-212/Arg-82 region with the extracellular channel in monomeric bR, was observed when several conditions were met: a), photoisomerization of retinal, b), protein flexibility, and c), Arg-82 side-chain down/up conformational change. As was noted in Baudry et al., and confirmed in subsequent quantum mechanical investigations of the photoevent, the nature of the potential, as well as the initial placement of water molecules and Arg-82, could influence the detailed timing of these results. Nevertheless, it was shown that water molecules could possibly move from a level below Arg-82 to the retinal binding site depending on the down-to-up movement of Arg-82 and the isomerization state of retinal. Another possibility suggested in Baudry et al. was the displacement of a water molecule (W4 in Baudry et al., corresponding to region III in Kandt et al.) to establish H-bond contact with Asp-85. In agreement with these findings, Kandt and co-workers report that water densities are highly sensitive to the conformation of Arg-82 and Schiff base protonation. When modeling the Schiff base deprotonation by neutralizing the retinal charge, Kandt et al. find that an upward movement of Arg-82 takes place, allowing a water rearrangement that connects densities IV (above Arg-82) and V (below Arg-82), linking the retinal to the extracellular bulk through a Grotthuss-like proton pathway.

#### ASP-96 AS AN INTRACELLULAR CHANNEL GATE

The results presented in Baudry et al. revealed a redistribution of external water molecules in the course of the PM simulation. Water molecules were found to penetrate the protein on the cytoplasmic side up to the level of Asp-96. It was concluded that Asp-96 was acting as a gate that defines the intra- and extramembrane region in the cytoplasmic side, controlling water penetration in the protein on the simulation timescale during the pump cycle. The results presented in Kandt et al. suggest the opening of a channel next to Asp-38 by which Asp-96 becomes accessible to intruding water. Interestingly, Kandt et al. find that this potential water access to Asp-96 can exist when using a probe slightly smaller than a water molecule, whereas Baudry et al. observed diffusion of intracellular water to the Asp-96 region. This difference may possibly originate from the difference in the systems modeled: the functional PM environment modeled in Baudry et al. has a thin layer of lipids between bR trimers, whereas

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the POPC/bR trimer simulated in Kandt et al. prevents bR trimers from getting close to each other and a priori modify the potential protein/protein and water/protein interactions present in the PM model. Despite these differences, the role of Asp-96 in gating a possible cytoplasmic water channel to the bulk is suggested in both studies.

## SCHIFF BASE/WATER INTERACTIONS

The stability of water 402's location in the crystal structure was investigated in Baudry et al. through free-energy perturbation studies that calculate the probability of finding a water in a given location during time-averaged "alchemical" perturbations, using a modification of the CHARMM force field parametrized to reproduce ab initio retinal/water interactions (Baudry et al., 2001, and references therein). The result described in Baudry et al. was that water 402, (labeled water W1 in Baudry et al., 2001), has an extremely high probability of existence of 0.99 around its crystallographic location. Through an interesting approach of averaged water density, Kandt et al. also find an average density of a water molecule at the water 402 crystallographic location. Both studies therefore suggested this particular water location to be stable. This is an important result, as the stability of water at this location has been shown in subsequent quantum mechanical/molecular mechanical studies to be force-field and water-model dependent (Hayashi et al., 2002).

## CONCLUSION

The work recently published by Kandt et al. introduces new approaches and presents new results to the exiting and developing field of integrated systems simulation. We emphasize here the similarities and complementarities between several key results published in Kandt et al. and previously in Baudry et al., as these similarities confirm the validity of various approaches to the study of bR. The two

studies differ in some details, for instance, in the extent of water diffusion in the cytoplasmic channel to Asp-96, the possible role of water in region III in bridging the Schiff base and Arg-82 to the extracellular channel, and the timescale of various events. At this time, it is not known whether these differences originate from artifacts in one or both of the simulations, or from the nature of the lipids and/or other parameters of the studies. However, both studies, in particular because of their converging results, demonstrate that simulation of integrated bR is a promising avenue for advancing our understanding of bR mechanisms.

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